Chapter 4:

Dimensions of Depression and Anxiety and the Hypothalamo-Pituitary-Adrenal Axis

Abstract

Background: Results on the association between depression and the HPA-axis have been inconsistent, possibly due to heterogeneity of the DSM-IV category of depression. Specific symptom-dimensions could be used as a more homogenous phenotype in HPA-axis research.

Methods: 1029 subjects with a lifetime depression and/or anxiety disorder from the NESDA study (mean age: 43.0±12.7; 67.4% female) provided 7 saliva samples to yield the cortisol awakening response (CAR), evening cortisol and dexamethasone suppression data. The dimensions of the tripartite model (General Distress, Anhedonic Depression and Anxious Arousal) were measured with the 30-item adapted Mood and Anxiety Symptoms Questionnaire (MASQ-D30) and analyzed in association with the cortisol measures using linear and non-linear regression.

Results: Median (interquartile range) scores of General Distress, Anhedonic Depression and Anxious Arousal were respectively 20 (14-27), 36 (28-44) and 15 (12-19), indicating large variability. Non-linear associations with the shape of an inverted U were found between General Distress, Anhedonic Depression and Anxious Arousal on one hand and total morning secretion and the dynamic of the CAR on the other hand. Both high and low severity levels were associated with a lower CAR, compared to intermediate levels of severity. Most of the associations remained significant when adjusted for covariates and the presence of DSM-IV diagnoses.

Conclusions: Non-linear associations were found between the CAR and the dimensions of the tripartite model. This could explain previous inconsistent findings regarding HPA-axis activity in depressed patients and illustrates the added value of symptom-dimensions for HPA-axis research.

Previously published as:

Wardenaar, KJ, Vreeburg, SA, van Veen, T, Giltay, EJ, Penninx, BWJH, Zitman, FG (2011). Dimensions of depression and anxiety and the Hypothalamo-Pituitary-Adrenal-axis. *Biological Psychiatry* 69: 366-373.

4.1 Introduction

Dysregulation of the Hypothalamo-Pituitary-Adrenal (HPA) axis may play a role in the etiology of depression (Holsboer & Barden, 1996; De Kloet, 2005). A hyperactive HPA-axis has indeed been found in severely depressed patients and, to a lesser extent, in outpatients and mildly depressed patients, measured through e.g. increased evening cortisol levels, a higher cortisol awakening response (CAR), an altered dexamethasone suppression test (DST) or a decreased response on the 'Dexamethasone Suppression Test' and the more sensitive 'Dexamethasone Corticotrophin Releasing Hormone (CRH) Suppression Test' (Bhagwaghar et al., 2005; Pruessner et al., 2003; Holsboer & Ising, 2010). Our group found a slightly higher CAR in subjects with a current or lifetime MDD and higher evening cortisol in subjects with a current MDD but found no differences in suppression on the DST (Vreeburg et al., 2009a). However, others have found no or other HPA-axis dysregulations in MDD outpatients (Stetler & Miller, 2005; Huber et al., 2006).

A possible explanation for these modest and inconsistent findings could be that the DSM-IV category of MDD is not an optimal clinical phenotype for HPA-axis research. Patients may even receive the same MDD diagnosis if they only overlap on one of nine criterion symptoms, resulting in a heterogeneous patient group. When comparing these patients to healthy controls, specific associations between symptoms and cortisol levels can easily go undetected. Similar problems arise with overall measures of 'depression severity', because persons with the same severity score can still have very different symptom patterns. There is much overlap in clinical features between MDD and anxiety disorders, leading to high comorbidity (Hasin et al., 2005), and both groups of disorders respond to similar treatments. This indicates that research should not be limited to MDD only, because a lot of underlying pathophysiology is likely to be shared with anxiety. Indeed, an altered HPA-axis has also been observed in anxious patients versus healthy controls (Schreiber et al, 1996; Erhardt et al., 2006, Abelson et al., 2007) and our research group found increased morning cortisol in current anxiety patients, mainly in current panic disorder with agoraphobia (Vreeburg et al., 2010). Research on the HPA-axis should thus address both specific and shared symptom domains of depression and anxiety.

A way to investigate symptom-specific associations with the HPA-axis can be through the use of s*ymptom-dimensions* as clinical determinants. A symptom dimension represents a continuum of increasing severity on a symptom-domain (Goldberg, 2000). Each dimension covers specific symptomatology, which can help to distinguish symptom-specific pathophysiological effects. In addition, dimensions are continuous, providing more statistical power to detect small -but potentially relevant- effects (MacCallum et al., 2002). Additionally, using continuous dimensions, non-linear (curved) associations can be effectively investigated. This is useful given the observations that both hypo-and hyperactivity of the HPA-axis are associated with higher risk of depression, indicating a non-linear, U-shaped association between HPA-axis activity and depression (Bremmer et al., 2007; Penninx et al., 2007).

A well-known dimensional model of anxiety and depression is the 'tripartite model' (Clark & Watson, 1991). In this model, a *'General Distress'* dimension covers symptoms of general psychological distress (e.g. pessimism and feelings of guilt), common to both depression and anxiety. In addition, a specific *'Anhedonic Depression'* dimension covers anhedonic symptoms (i.e. lack of positive affect and emotionality), associated with depression. An *'Anxious Arousal'* dimension covers symptoms of somatic hyperarousal (e.g. sweating, trembling and palpitations), associated rather specifically with anxiety/panic disorder. Several studies have found this model to work well in different patient populations (Keogh & Reidy, 2000; Marshall et al., 2003; de Beurs et al., 2007; Wardenaar et al., 2010). The association between the tripartite dimensions and the HPA-axis was investigated by our group (Veen et al., 2011). We recently found that General Distress and Anhedonic Depression were both associated with morning cortisol. These associations had an "inverted U-shape": low and high dimensional scores were associated with decreased morning cortisol. This observation can explain why low and high HPA-axis activity is observed in depressed patients depending on their specific profile of symptomatology, which could cause the inconsistencies in the literature so far. Using the dimensions of the tripartite model can thus have added value for HPA-axis research.

Therefore, the present study aimed to answer several questions. First, we investigated whether the dimensions of the tripartite model were associated with the HPA-axis in a large sample of lifetime depression and/or anxiety patients (n=1029). Second, we investigated the shape of these associations. Third, we investigated whether the dimensional associations were generalizable across different DSM-IV groups (e.g. current versus lifetime patients) and whether they provided additional information about HPA-axis variability on top of the DSM-IV diagnoses that were previously found to be associated with the HPA-axis (e.g. lifetime/current MDD, current anxiety).

4.2 Methods and Materials

Subjects

Subjects came from the NESDA study, a large longitudinal study to investigate the course of depressive and anxiety disorders. The NESDA sample consists of 2.981 subjects (mean age 41.9; 1.002 men and 1.979 women), who were recruited from community, primary care and specialized mental health care organizations. The sample consists of 2.329 subjects with a lifetime diagnosis of depressive or anxiety disorder and 652 subjects without a lifetime psychiatric diagnosis. Detailed objectives and rationales can be found elsewhere (Penninx et al., 2008). The protocol of the NESDA study was approved centrally by the Ethical Review Board of the Leiden University Medical Center and by local review boards of the participating centres. All participants signed informed consent.

Of the 2981 subjects in NESDA, 2167 (72.6%) returned saliva samples. These subjects were older ($p<0.001$) and had more years of education ($p<0.001$) than the subjects who did not return the saliva samples. 130 subjects were excluded because they

used corticosteroids, 19 because they were pregnant or breastfeeding and 50 subjects using tricyclic antidepressants (TCA; WHO Anatomical Therapeutic Chemical classification N06AA) because TCA's were shown to affect the HPA-axis in previous research (6). Users of selective serotonin reuptake inhibitors (SSRIs; N06AB) and other antidepressants (N06AF, N06AG and N06AX) were *not* excluded. Of the remaining 1968 subjects, 1782 subjects returned the required questionnaires. These were significantly younger ($p=0.03$) and had more years of education ($p<0.001$) than subjects who did not return all questionnaires. During data cleaning (described below), 278 subjects were excluded, resulting in a group of 1378 subjects with usable cortisol samples. Of these, 1029 subjects had a lifetime depression and/or anxiety disorder and were used as study group for the main analyses.

Salivary Cortisol Measurements

An extensive description of the cortisol measurement and analysis was presented previously (6). Participants were instructed to collect saliva samples at home on a regular working day. Saliva was collected with Salivettes® (Starstedt AG, Germany) at seven sampling points. The CAR was assessed with four time points: at awakening (T1), 30 (T2), 45 (T3) and 60 minutes later (T4). Two samples were collected at 22h00 (T5) and 23h00 (T6) to assess the (basal) evening cortisol level. Directly after T6, participants ingested 0.5 mg of dexamethasone and the next morning saliva was collected at awakening (T7). Samples were centrifuged at 2000g (for 10 min), aliquoted and stored at -80°C. The analysis of cortisol was done with competitive electrochemiluminescence immunoassay (E170, Roche, Switzerland) (van Aken et al., 2003). The functional detection limit was 2.0 nmol/l and the intra- and interassay variability coefficients were less than 10% in the measurement range.

During data cleaning, 149 subjects who collected their cortisol samples more than five minutes before or after the right protocol time were excluded. Also, 129 subjects were excluded because they had cortisol samples with values higher than two standard deviations above the mean. These values exceeded the realistic range for saliva cortisol and were likely to be a result of measurement factors (e.g. bleedings of the gums after tooth brushing or as a result of gingivitis). Three cortisol indicators were computed: the CAR, the evening cortisol level and the DST. The CAR was assessed by calculation of the area under the curve with respect to the ground (AUCg) and with respect to the increase (AUCi), by use of a trapezoid formula (Pruessner et al., 2003). The AUCg estimates the total body exposure to cortisol and predicts mean saliva cortisol exposure throughout the day. The AUCi is a measure of the dynamic of the CAR, related to the sensitivity of the system and change in cortisol exposure over time (Pruessner et al., 2003; Edwards et al., 2001). The mean of cortisol levels at T5 and T6 was calculated as a measure of evening cortisol. The DST was assessed using the samples at T1 and T7. The percentage of suppression by dexamethasone was calculated by taking the ratio of T1/T7, with higher values indicating more post-dexamethasone suppression.

Psychopathology

All participants completed the shortened, 30-item, Dutch adaptation of the MASQ (Watson et al., 1995a,b: the MASQ-D30 (Wardenaar et al., 2010). In the MASQ-D30, individuals rate how much in the past week they have experienced "feelings, sensations, problems and experiences that people sometimes have" on a 5-point scale, with 1 being "not at all" and 5 being "extremely". The MASQ-D30 consists of three 10-item subscales that measure General Distress, Anhedonic Depression and Anxious Arousal and has good psychometric characteristics (Wardenaar et al., 2010). The Composite International Diagnostic Interview (CIDI, WHO version 2.1) was used to assess the DSM-IV criteria for depressive disorders (i.e. MDD and dysthymia) and anxiety disorders (i.e. panic disorder, social phobia, generalized anxiety disorder and agoraphobia).

Covariates

Sociodemographic variables (gender, age), sampling factors (time of awakening, working status, seasonality and sleep duration) and physical health indicators (smoking, alcohol use/dependence, physical activity, cardiovascular disease [CVD]) have been found to be associated with salivary cortisol levels in previous research using the NESDA data (Vreeburg et al., 2009b). These determinants were treated as covariates in the present analyses. Each participant reported time of awakening and working status. The month of cortisol collection was dichotomized into months with less (October – February) versus more (March – September) daylight. Sleep duration was dichotomized as more or less than 6 hours/night. Smoking was dichotomized into current and non-smokers. The International Physical Activity Questionnaire (IPAQ) was used to assess physical activity, expressed in 100 MET-minutes (metabolic equivalent of number of calories spent per minute) per week (Craig et al., 2003). Prevalent CVD was established using an algorithm based on self-report and medication use. The Alcohol Use Disorder Identification Test (Saunders et al., 1993) was used to assess the number of daily ingested alcoholic beverages and the presence of alcohol dependence (a score >14 for males and >12 for females).

Statistical Analyses

The AUCs and the DST (T1/T7) were log-transformed to improve normality. Inspection of the plotted standardized residuals and normal (P-P) plots of all univariate and multivariate models revealed that the residuals were normally distributed.

To investigate the associations of the dimensions with cortisol exposure, several regression analyses were conducted. In each analysis, one of the dimensions was the continuous predictor variable and a cortisol indicator the outcome variable. Next, to test if the association had the shape of a curve instead of a straight line, a quadratic term of the scale was added as predictor variable (e.g. both General Distress and [General Distress]^2); If the regression coefficients were significant for both the linear and quadratic

term, the association with cortisol would have a curved shape. All analyses were conducted without (Crude) and with covariates (Model 1). Finally, lifetime MDD, current (6-month) MDD, and current anxiety were added as dichotomous covariates (Model 2). If the regression coefficients of the dimension did not change in this incremental model, this would indicate that the dimension explained variation in the cortisol indicator, independently from DSM-IV status. For each model, the proportion of explained variance was calculated (R^2). The variance inflation factor (VIF) was calculated to check for collinearity. Only in the non-linear models, the VIF indicated collinearity between the dimension and its quadratic term, which is a well-documented phenomenon for polynomial regression models (33). This collinearity was not likely to affect the reliability of our results, since the collinear variables are mathematically related to each other and not intended as independent predictors. Moreover, eliminating the collinearity by centering the linear and non-linear terms (Brauner & Shacham, 1998; data not shown) did not lead to large changes in the observed results, which further indicated that collinearity did not affect our findings. Durbin-Watson coefficients were calculated to test for auto-correlated residuals. For all models, the coefficients (range: 1.96-2.03) suggested to reject the hypothesis of auto-correlated residuals (Savin & White, 1977). P-values <0.05 were considered significant. Because we tested only a priori hypothesized associations and for confounding, we did not correct for multiple testing.

Additional analyses were conducted to evaluate to what extent the associations were generalizable across different diagnostic groups (healthy, remitted patients and current patients). To investigate whether the inclusion of remitted patients in the main research group, all analyses were rerun in a group of current patients only (n=729). To investigate whether the associations could be generalized across the complete spectrum of diagnostic severity (from healthy to ill), all analyses were rerun in a group including lifetime patients and healthy subjects (n=1378). All analyses were done with SPSS 16.0.

4.3 Results

Demographic information and diagnoses

The demographic and diagnostic information of the study group is shown in Table 4.1. The mean age was 43.0 years (SD=12.7) and the percentage of women was 67.4%. Of the subjects, 45.6% had a current and 24.7% had a remitted MDD diagnosis with or without a comorbid anxiety disorder. In addition, 25.3% had a current and 4.4% had a remitted anxiety diagnosis without MDD. Of the subjects 19.8% used SSRIs and 8.0% used other antidepressants.

General Distress had a median of 20 (interquartile range [IQR]: 14-26; Anhedonic Depression had a median of 36 (IQR: 29-43); and Anxious Arousal had a median of 15 (IQR: 12-18), which indicated that there was considerable variability on each of the dimensions.

The cortisol awakening response: AUCg and AUCi

None of the AUC's showed a linear association with any of the symptom-dimensions (Table 4.2). The results of the regression analyses with the added quadratic terms are shown in Table 4.3. The AUCg showed a significant curved association with Anhedonic Depression. The AUCi showed significant curved associations with General Distress, Anhedonic Depression and Anxious Arousal. All associations remained statistically significant when covariates were added (Model 1 in Table 4.3). In addition, when lifetime MDD, current MDD and current anxiety disorders were added as covariates (Model 2 in Table 4.3), all associations remained significant and regression coefficients barely changed (1.6 to 6.0%). This indicated that the curve-shaped associations explain variation in the AUC's, independently from lifetime and current DSM-IV diagnoses.

R^2-statistics indicated that 6.7 to 10.6% of the variance in the AUCs was explained by the different multivariate regression models. The dimensions alone explained 0.8 to 1.0% of the variance in the AUCs, which indicated a small effect size, but these percentages were considerably more substantial for each individual dimension than for lifetime MDD, current MDD and Current Anxiety together, which only added 0.2-0.6% of explained variance in the AUCs (Model 2 compared to Model 1 in Table 4.3). For all analyzed associations, the regression coefficient was positive for the linear term and negative for the quadratic term of the dimension. In other words, the associations had an inverted U-shape. The AUC first increased with increasing dimensional score, then slowly flattened and eventually decreased at the severe end of the dimension (see Figure 4.1; to aid interpretation of the figure, categorized dimensions are depicted on the x-axis).

When the non-linear associations between each dimension and the AUCs were additionally adjusted for the other two dimensions, the results remained the same (data not shown). This indicates that the observation of similarly shaped associations between each of the dimensions and the AUCs were not merely an artefact of (linear) correlations between the dimensions (ρ=0.44-0.66 in the current research group).

In the research group with only current patients (n=729; see Supplementary Table 4.4 [S1]), the AUCi showed significant curved associations with General Distress, Anhedonic Depression and Anxious Arousal. The AUCg showed a borderline significant curved association with Anhedonic Depression. These results were largely similar to the main results; they hardly changed after excluding remitted patients. In the research group with lifetime patients and healthy subjects (n=1378; Supplementary Table 4.5 [S2]), the AUCi also showed curved associations with General Distress, Anhedonic Depression and Anxious Arousal. The AUCg showed a curved association with General Distress and Anhedonic Depression. These results hardly differed from the main results. The curved associations thus seem to be generalizable across the complete spectrum of healthy subjects and current and remitted patients.

Table 4.1: Characteristics of the study group

Characteristic	Total
N	1029
% female	67.4%
Age (Mean, SD)	43.0 (12.7)
% working on sampling day	60.4%
% sampling in light month	57.6%
% < 6 hours of sleep	30.5%
% smoking	33.9%
Physical activity (mean 1000 MET-min/week; SD)	3.5 (3.0)
Current (6-month) MDD and/or dysthymia	178 (17.3%)
Current (6-month) anxiety disorder	260 (25.3%)
Current (6-month) MDD and/or dysthymia with comorbid anxiety disorder	291 (28.3%)
Remitted (6-month) MDD and/or dysthymia	172 (16.7%)
Remitted (6-month) anxiety disorder	45 (4.4%)
Remitted (6-month) MDD and/or dysthymia with comorbid anxiety disorder	83 (8.0%)
Medication use	
SSRI	19.8%
Other antidepressants	8.0%
Median MASQ scale scores (median and interquartile range)	
- General Distress	20 (14-26)
- Anhedonic Depression	36 (29-43)
- Anxious Arousal	15 (12-18)
Cortisol measurements, mean (SD)	
Cortisol T1, at awakening (nmol/l)	16.4 (6.0)
Cortisol T2, + 30 min (nmol/l)	21.0 (8.7)
Cortisol T3, + 45 min (nmol/l)	19.6 (9.4)
Cortisol T4, + 60 min (nmol/l)	17.1 (8.2)
AUCg (nmol/l/h)[1]	17.8 (17.4-18.2)
AUCi (nmol/l/h)[1]	2.4 (2.0-2.8)
Cortisol T5, at 10pm (nmol/l)	5.2 (2.9)
Cortisol T6, at 11pm (nmol/l)	5.1 (3.0)
Evening cortisol (T5+T6 / 2)	5.1 (2.6)
Cortisol T7, at awakening the next day (nmol/l)	6.9 (3.5)
mean Cortisol Suppression Ratio[1,2]	2.46 (2.39-2.54)

Table 4.1 (continued). Legend: AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; MDD = major depressive disorder; MET-min = metabolic equivalent of number of calories spent per minute; SSRI = selective serotonin reuptake inhibitor; T1 to T7 = 7 time points of salivary cortisol collection;
[1] Because of their skewed distributions, back-transformed geometric mean and 95% confidence intervals are presented.
[2] Cortisol suppression ratio = log (salivary cortisol level at T1/salivary cortisol level at T7)

Evening cortisol levels and the DST

General Distress showed a linear association with the DST, which remained significant after addition of covariates (Model 1), and current and lifetime MDD and current anxiety (Model 2). However, when remitted MDD patients were excluded from the study group, the association was no longer significant, indicating that the association between general distress and the DST was partly explained by diagnostic status. Anhedonic Depression and Anxious Arousal were not associated with the DST. None of the dimensions was associated with evening cortisol.

4.4 Discussion

The present study investigated the associations between several HPA-axis indicators and the dimensions of the tripartite model in a large group of psychiatric outpatients with a lifetime depression and/or anxiety disorder. Analyses with the AUCg and AUCi showed the dimension Anhedonic Depression to be associated with both total cortisol exposure and the dynamic of the CAR. The dimensions General Distress and Anxious Arousal were only associated with the dynamic of the CAR. Notably, the associations had the curved shape of an inverted U: both low and high dimensional scores were associated with a lower morning cortisol exposure, compared to intermediate dimension scores. Importantly, each individual dimension explained more variation in morning cortisol exposure than was explained by lifetime MDD, current MDD, and current anxiety disorders together. Interestingly, largely similar associations were found when only patients with a current diagnosis were included in the analyses and when lifetime patients and healthy subjects were analysed together. This indicates that the identified associations are not limited to (current) psychiatric patients only, but can be generalized to a broader group, including remitted patients and healthy subjects. Evening cortisol and the DST did not show any consistent associations with the tripartite dimensions.

Table 4.2: Linear associations between the MASQ-D30 dimensions and cortisol indices in 1029 subjects with lifetime psychopathology.

Dimension		Log AUCg	R^2	Log AUCi	R^2	Evening cortisol	R^2	DST	R^2
General Distress (GD)	Crude	0.03 (0.28)	0.001	0.05 (0.14)	0.002	-0.02 (0.62)	0.000	0.05 (0.10)	0.003*
	Model 1	0.05 (0.08)	0.101	0.04 (0.17)	0.057	-0.03 (0.24)	0.176	0.06 (0.04)	0.046*
	Model 2	0.04 (0.16)	0.107	0.04 (0.23)	0.060	-0.05 (0.13)	0.182	0.07 (0.026)	0.049*
Anhedonic Depression (AD)	Crude	0.04 (0.26)	0.001	0.03 (0.42)	0.001	0.03 (0.29)	0.001	0.00 (0.97)	0.000
	Model 1	0.03 (0.30)	0.099	0.03 (0.36)	0.056	0.00 (0.99)	0.174	0.03 (0.36)	0.043
	Model 2	0.02 (0.45)	0.106	0.02 (0.51)	0.059	-0.01 (0.77)	0.180	0.04 (0.28)	0.045
Anxious Arousal (AA)	Crude	0.02 (0.45)	0.001	0.03 (0.34)	0.001	0.02 (0.53)	0.000	0.01 (0.75)	0.000
	Model 1	0.03 (0.26)	0.100	0.03 (0.37)	0.056	0.00 (0.91)	0.174	0.03 (0.38)	0.043
	Model 2	0.02 (0.45)	0.106	0.02 (0.48)	0.059	0.00 (0.94)	0.180	0.04 (0.27)	0.045

Data are β-coefficients (p-value); AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; DST = dexamethasone suppression test

Model 1 is adjusted for sociodemographic factors (sex, age and Northern European ancestry), sampling factors (working, time of awakening, sleep duration and months with more or less daylight), and health indicators (smoking, alcohol use (# of daily beverages), alcohol dependence, physical activity, cardiovascular disease). Model 2 is additionally adjusted for current major depressive disorder and current anxiety disorder.

*) When remitted MDD patients were removed, these associations were no longer significant

Table 4.3: Non-linear associations between the MASQ-D30 dimensions and cortisol indices in 1029 subjects with lifetime psychopathology.

Scale		Term	Log AUCg	R^2	Log AUCi	R^2	Evening cortisol	R^2	DST	R^2
General Distress (GD)	Crude	Linear (GD)	0.29 (0.09)	0.003	0.51 (0.003)	0.010	-0.15 (0.36)	0.00	0.08 (0.64)	0.003
		Quadratic (GD^2)	-0.26 (0.13)		-0.47 (0.005)		0.14 (0.41)	1	-0.02 (0.84)	
	Model 1	Linear (GD)	0.34 (0.03)	0.104	0.50 (0.003)	0.064	-0.17 (0.28)	0.17	0.09 (0.57)	0.046
		Quadratic (GD^2)	-0.29 (0.07)		-0.46 (0.006)		0.13 (0.39)	6	-0.03 (0.86)	
	Model 2	Linear (GD)	0.37 (0.03)	0.111	0.47 (0.005)	0.066	-0.15 (0.35)	0.18	0.12 (0.49)	0.049
		Quadratic (GD^2)	-0.32 (0.06)		-0.44 (0.008)		0.10 (0.51)	2	-0.05 (0.78)	
Anhedonic Depression (AD)	Crude	Linear (AD)	0.73 (0.002)	0.010	0.66 (0.005)	0.008	-0.06 (0.82)	0.00	0.03 (0.91)	0.000
		Quadratic (AD^2)	-0.70 (0.003)		-0.64 (0.007)		0.09 (0.71)	1	-0.03 (0.90)	
	Model 1	Linear (AD)	0.63 (0.006)	0.106	0.59 (0.011)	0.062	-0.18 (0.41)	0.17	0.10 (0.67)	0.043
		Quadratic (AD^2)	-0.60 (0.008)		-0.57 (0.014)		0.18 (0.40)	5	-0.07 (0.76)	
	Model 2	Linear (AD)	0.62 (0.006)	0.112	0.57 (0.016)	0.064	-0.13 (0.56)	0.18	0.13 (0.57)	0.045
		Quadratic (AD^2)	-0.61 (0.008)		-0.55 (0.019)		0.12 (0.58)	0	-0.10 (0.67)	
Anxious Arousal (AA)	Crude	Linear (AA)	0.27 (0.12)	0.003	0.51 (0.003)	0.009	0.04 (0.83)	0.00	0.09 (0.59)	0.000
		Quadratic (AA^2)	-0.25 (0.14)		-0.49 (0.004)		-0.02 (0.91)	0	-0.08 (0.63)	
	Model 1	Linear (AA)	0.28 (0.09)	0.102	0.51 (0.003)	0.064	-0.12 (0.46)	0.17	0.18 (0.29)	0.043
		Quadratic (AA^2)	-0.25 (0.13)		-0.48 (0.004)		0.12 (0.46)	5	-0.16 (0.36)	
	Model 2	Linear (AA)	0.26 (0.13)	0.108	0.48 (0.005)	0.066	-0.10 (0.55)	0.18	0.22 (0.21)	0.046
		Quadratic (AA^2)	-0.24 (0.16)		-0.46 (0.007)		0.09 (0.56)	0	-0.19 (0.28)	

Data are β-coefficients (p-value); AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; DST = dexamethasone suppression test. Model 1 is adjusted for sociodemographic factors (sex, age and Northern European ancestry), sampling factors (working, time of awakening, sleep duration and months with more or less daylight), and health indicators (smoking, alcohol use (# of daily beverages), alcohol dependence, physical activity, cardiovascular disease). Model 2 is additionally adjusted for lifetime and current major depressive disorder and current anxiety.

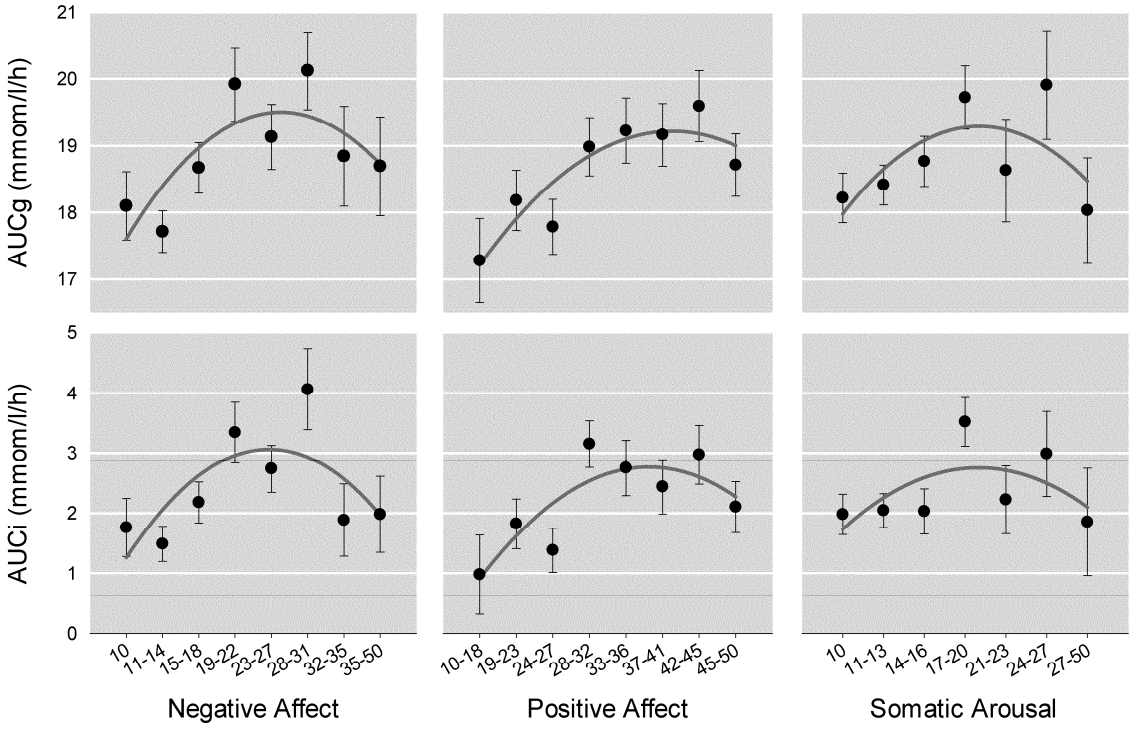

Figure 4.1: Plots of the association between each tripartite model dimension (x-axis) and the area under the curve with respect to the ground (AUCg, upper plots y-axis) and the area under the curve with respect to the increase (AUCi, lower plots y-axis) of the cortisol morning response. Both AUCs had a slightly skewed distribution and were log-transformed before analysis; displayed are the back-transformed means and standard errors. Categorized dimensions are depicted on the x-axis to aid interpretability. The categorized dimensions on the x-axis were only used to aid clearer interpretability of the figure; the tripartite dimensions were actually treated as continuous variables in all analyses.

These results have some interesting implications. Change in HPA-axis activity seems not to be exclusively linked to DSM-IV diagnosis, but also to specific symptom patterns and severity. For instance, the association of morning cortisol exposure with Anhedonic Depression across subjects with lifetime MDD, current MDD and/or current anxiety (treated as covariates), indicates that it is not merely the presence of a diagnosis, but also the amount of, for instance, associated Anhedonic Depression that predicts the height and shape of cortisol exposure during the CAR. Thus, etiological research with the HPA-axis ought not to be limited to the specific group of MDD patients, because the HPA-axis is very likely to play a broader role, for instance in the etiology of subtreshold depression and anxiety. From this perspective, the present results can be regarded as an elaboration

on previous findings of increased morning cortisol in lifetime MDD and anxiety (Vreeburg et al., 2009a; 2010).

Our use of a dimensional approach enabled us to detect non-linear associations that would have gone undetected, had we only used DSM-IV diagnoses. The observed 'inversed U'-shaped associations between the CAR indicators and the dimensions replicates earlier findings by our group in another, smaller sample (Veen et al., 2011) and indicates that, depending on his/her dimensional symptom profile, a patient is more likely to have higher or lower exposure to cortisol during the morning. This could explain why both hypo- and hypercortisolemia are observed in depressed (elderly) patients (Bremmer et al., 2007; Penninx et al., 2007). A possible explanation for the inverted U-shape of the observed associations is that cortisol levels increase with dimensional symptom severity until a critical threshold is reached and the HPA-axis is down-regulated or exhausted (Veen et al., 2011). There are several potential underlying mechanisms for such "hypocortisolism" (Heim et al., 2000). It could be related to a down-regulation of CRH receptors in the pituitary, following a longer period of stress-induced hypothalamic CRH secretion, resulting in lower adrenocorticotropic hormone (ACTH) and reduced cortisol levels. Other possible mechanisms that have found some support from studies in both humans and animals could be a reduced biosynthesis or depletion of CRH, ACTH, cortisol (Heim et al., 2000) or increased sensitivity of the HPA-axis to negative feedback (Holsboer et al., 1985). Although some studies found that especially patients with severe (psychotic) major depression showed a higher cortisol exposure during the CAR versus healthy controls (Belanoff et al., 2001; Posener et al., 2000), other studies in humans and animals have found the HPA-axis to be down-regulated in response to prolonged severe stress, leading to a blunted CAR (Oldehinkel et al., 2001; Meinlschmidt et al., 2005). Our results fit in with both lines of evidence and suggest that they are not necessarily inconsistent.

The association between General Distress and Anhedonic Depression on the one hand and the HPA-axis on the other hand, has been investigated in previous studies outside the tripartite framework (using various different questionnaires). Several studies found an association between measures of General Distress (also called 'Negative Affect') and increased HPA-axis activity in healthy adults (van Eck et al., 1996; Smyth et al., 1998; Buchanan et al., 1999; Jacobs et al., 2007; Polk et al., 2005). Our findings are in line with this, since we also found an increase of the CAR when scores increased within the lower (healthy) spectrum of General Distress. Previous studies mainly investigated the association between the HPA-axis and the opposite pole of Anhedonic Depression, called 'Positive Affect'. These studies generally found decreasing cortisol levels with increasing Positive Affect (Smyth et al., 1998; Polk et al., 2005; La et al., 2005; Steptoe et al., 2008). Our findings are in line with this, because we found an increase in cortisol exposure during the CAR with increasing Anhedonic Depression (i.e. *lack of* Positive Affect). The only previous study to investigate the association between Anxious Arousal and (morning) cortisol (n=36) did not find an association (Veen et al., 2011). However, larger statistical power due to the much larger sample size in the present study (n=1029) could explain

why we did find an association between Anxious Arousal and the dynamic of morning cortisol exposure. Interestingly, our observed U-shaped associations were not found by any of the abovementioned studies, possibly because they used healthy subjects with relatively low symptom severity and were thus unable to detect a decrease in subjects with severe symptomatology, or because only linear associations were explored.

Our findings could be regarded as further (external) validation of the dimensions of the tripartite model and the MASQ-D30. The associations of the MASQ-D30 scales with different aspects of the CAR indicate that they are not merely psychometric constructs, but also have a biological substrate. The tripartite dimensions (as measured with the MASQ-D30) could thus be a promising clinical phenotype for future etiological research.

The present study has some strong characteristics. First, the studied group was one of the largest to date, which increased the reliability of our results. Second, we were able to test the associations across groups including current and remitted MDD patients, anxiety patients and healthy subjects, making our results broadly generalizable. Third, a wide range of determinants of the HPA-axis (Vreeburg et al., 2009b) were considered as possible confounders. Fourth, we studied multiple cortisol indicators that were all indicative of different aspects of the HPA-axis across the day. The present results should also be interpreted in the light of some limitations. First, our results are cross-sectional and cannot indicate change over time or causality. Second, the saliva samples were collected by the participants themselves at home and compliance with the sampling protocol was not monitored. This may have resulted in some measurement error (Vreeburg et al., 2009a). Third, we only measured cortisol during one day, possibly missing day-to-day variations in cortisol levels, which could have further increased measurement error. Fourth, systematic differences between those that did and did not return saliva samples may have biased our results somewhat towards an older and higher educated population. Fifth, our results apply to outpatients with relatively low levels of symptom severity, which limits generalizability of our results to severely ill psychiatric inpatients. Sixth, we only used three, quite broad symptom-dimensions, whereas in reality more (sub)dimensions may exist (Hollander-Gijsman et al., 2010). Finally, the effect sizes suggest that many more factors play a role in symptom dimensions of depression and anxiety on top of cortisol, which, for now, prevents the use of cortisol measurements as clinical marker for psychopathology in individual patients.

In future research, a prospective design should be used to investigate the association between symptom-dimensions and cortisol indicators across a large range of clinical patients (from subtreshold to inpatient), using a broad range of symptom dimensions.

In conclusion, the dimensions of the tripartite model were found to be associated with morning cortisol exposure. Because the dimensions were continuous, non-linear associations could be detected, demonstrating the added value of symptom-dimensions when investigating the role of small and/or complex neuroendocrine mechanisms, underlying psychiatric disease.

Supplementary Table 4.4 (S1): Non-linear associations between the MASQ-D30 dimensions and cortisol indices in 729 subjects with a current major depressive disorder and/or an anxiety disorder.

Scale		Term	AUCg	R^2	AUCi	R^2	Evening cortisol	R^2	DST	R^2
General Distress (GD)	Crude	Linear (GD)	0.39 (0.06)	0.006	0.60 (0.003)	0.012	-0.10 (0.63)	0.001	-0.05 (0.83)	0.001
		Quadratic (GD^2)	-0.35 (0.09)		-0.58 (0.005)		0.12 (0.58)		0.08 (0.69)	
	Model 1	Linear (GD)	0.39 (0.05)	0.105	0.59 (0.003)	0.065	-0.20 (0.30)	0.189	-0.04 (0.87)	0.040
		Quadratic (GD^2)	-0.34 (0.09)		-0.56 (0.006)		0.18 (0.34)		0.08 (0.89)	
	Model 2	Linear (GD)	0.38 (0.06)	0.110	0.59 (0.004)	0.069	-0.23 (0.23)	0.196	-0.03 (0.89)	0.050
		Quadratic (GD^2)	-0.34 (0.08)		-0.55 (0.006)		0.19 (0.32)		0.07 (0.73)	
Anhedonic Depression (AD)	Crude	Linear (AD)	0.68 (0.020)	0.008	0.70 (0.016)	0.008	-0.04 (0.89)	0.002	-0.07 (0.82)	0.000
		Quadratic (AD^2)	-0.65 (0.027)		-0.70 (0.017)		0.08 (0.78)		0.07 (0.80)	
	Model 1	Linear (AD)	0.50 (0.08)	0.103	0.59 (0.042)	0.059	-0.19 (0.49)	0.188	0.00 (0.99)	0.039
		Quadratic (AD^2)	-0.50 (0.08)		-0.59 (0.044)		0.19 (0.49)		0.03 (0.93)	
	Model 2	Linear (AD)	0.54 (0.06)	0.110	0.58 (0.045)	0.063	-0.15 (0.59)	0.194	0.02 (0.94)	0.048
		Quadratic (AD^2)	-0.55 (0.06)		-0.58 (0.048)		0.13 (0.64)		0.00 (0.99)	
Anxious Arousal (AA)	Crude	Linear (AA)	0.44 (0.04)	0.007	0.53 (0.011)	0.009	0.08 (0.72)	0.002	0.10 (0.65)	0.000
		Quadratic (AA^2)	-0.40 (0.06)		-0.53 (0.012)		-0.03 (0.88)		-0.09 (0.68)	
	Model 1	Linear (AA)	0.45 (0.03)	0.106	0.54 (0.010)	0.062	-0.07 (0.73)	0.189	0.18 (0.40)	0.039
		Quadratic (AA^2)	-0.40 (0.05)		-0.54 (0.010)		0.09 (0.64)		-0.16 (0.46)	
	Model 2	Linear (AA)	0.40 (0.06)	0.110	0.55 (0.010)	0.066	-0.06 (0.76)	0.194	0.21 (0.33)	0.050
		Quadratic (AA^2)	-0.36 (0.08)		-0.54 (0.011)		0.08 (0.67)		-0.19 (0.38)	

Data are β-coefficients (p-value); AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; DST = dexamethasone suppression testModel 1 is adjusted for sociodemographic factors (sex, age and Northern European ancestry), sampling factors (working, time of awakening, sleep duration and months with more or less daylight), and health indicators (smoking, alcohol use (# of daily beverages), alcohol dependence, physical activity, cardiovascular disease). Model 2 is additionally adjusted for lifetime and current major depressive disorder and current anxiety disorder.

Supplementary Table 4.5 (S2): Non-linear associations between the MASQ-D30 dimensions and cortisol indices in 1378 subjects with and without lifetime psychopathology

Scale		Term	AUCg	R^2	AUCi	R^2	Evening cortisol	R^2	DST	R^2
General Distress (GD)	Crude	Linear (GD)	0.40 (0.005)	0.009	0.47 (0.001)	0.011	0.01 (0.95)	0.000	0.13 (0.38)	0.003
		Quadratic (GD^2)	-0.33 (0.02)		-0.41 (0.004)		0.01 (0.92)		-0.08 (0.58)	
	Model 1	Linear (GD)	0.44 (0.001)	0.100	0.43 (0.002)	0.067	-0.04 (0.78)	0.165	0.13 (0.36)	0.043
		Quadratic (GD^2)	-0.36 (0.009)		-0.37 (0.008)		0.03 (0.85)		-0.07 (0.63)	
	Model 2	Linear (GD)	0.40 (0.005)	0.105	0.37 (0.011)	0.068	-0.06 (0.66)	0.170	0.20 (0.18)	0.045
		Quadratic (GD^2)	-0.34 (0.015)		-0.33 (0.021)		0.03 (0.84)		-0.12 (0.43)	
Anhedonic Depression (AD)	Crude	Linear (AD)	0.55 (0.004)	0.008	0.56 (0.004)	0.008	-0.04 (0.85)	0.003	0.18 (0.37)	0.001
		Quadratic (AD^2)	-0.50 (0.010)		-0.51 (0.009)		0.09 (0.65)		-0.16 (0.41)	
	Model 1	Linear (AD)	0.43 (0.022)	0.094	0.47 (0.013)	0.064	-0.20 (0.27)	0.165	0.23 (0.23)	0.042
		Quadratic (AD^2)	-0.38 (0.043)		-0.43 (0.025)		0.20 (0.28)		-0.18 (0.35)	
	Model 2	Linear (AD)	0.39 (0.038)	0.102	0.45 (0.043)	0.067	-0.18 (0.32)	0.170	0.27 (0.17)	0.044
		Quadratic (AD^2)	-0.37 (0.050)		-0.41(0.041)		0.16 (0.38)		-0.21 (0.29)	
Anxious Arousal (AA)	Crude	Linear (AA)	0.36 (0.015)	0.006	0.48 (0.001)	0.009	0.19 (0.19)	0.003	0.15 (0.31)	0.001
		Quadratic (AA^2)	-0.31 (0.034)		-0.44 (0.003)		-0.15 (0.32)		-0.14 (0.34)	
	Model 1	Linear (AA)	0.35 (0.014)	0.095	0.45 (0.002)	0.066	0.02 (0.89)	0.165	0.21 (0.15)	0.041
		Quadratic (AA^2)	-0.30 (0.036)		-0.42 (0.004)		-0.01 (0.96)		-0.19 (0.20)	
	Model 2	Linear (AA)	0.27 (0.06)	0.102	0.45 (0.002)	0.066	0.01 (0.94)	0.169	0.26 (0.08)	0.042
		Quadratic (AA^2)	-0.24 (0.09)		-0.42 (0.004)		-0.01 (0.97)		-0.22 (0.13)	

Data are β -coefficients (p-value); AUCg = area under the curve with respect to the ground; AUCi = area under the curve with respect to the increase; DST = dexamethasone suppression test. Model 1 is adjusted for sociodemographic factors (sex, age and Northern European ancestry), sampling factors (working, time of awakening, sleep duration and months with more or less daylight), and health indicators (smoking, alcohol use (# of daily beverages), alcohol dependence, physical activity, cardiovascular disease). Model 2 is additionally adjusted for lifetime and current major depressive disorder and current anxiety disorder.